

In re the Divisional Patent Application:

LOW OXYGEN AFFINITY MUTANT HEMOGLOBINS

Inventors: Chien Ho, Ching-Hsuan Tsai, Tsuei-Yun Fang and Tong-Jian Shen

Filed: November 9, 2001

Serial No.: 09/986,666

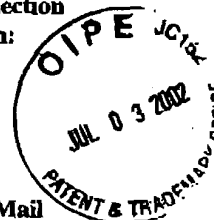
Attorney Docket No.: 002547/20118/div3

Transmitted herewith are the following items in connection
with the above-identified divisional patent application:

- Preliminary Amendment (10 pp)

Dated: July 3, 2002

The stamp of the U.S. Patent and Trademark Office Mail
Room hereon acknowledges the receipt of the above-identified
papers on the date indicated by such stamp.



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of)	
CHIEN HO, et al.)	Group Art Unit: 1653
Serial No.: 09/986,666)	
Filed: November 9, 2001)	Examiner: Karen C. Carlson
For: LOW OXYGEN AFFINITY MUTANT)	
HEMOGLOBINS)	
Attorney Docket No.:)	
002547/20118/div3)	

PRELIMINARY AMENDMENT

Commissioner for Patents
BOX NON-FEE AMENDMENT
Washington, D.C. 20231

Dear Sir:

This is in response to the "Notice of Non-Compliant Amendment (37 CFR 1.121)" mailed June 6, 2002, to which a one-month time limit for response was set. The preliminary amendment filed on November 9, 2001 did not comply with revised 37 CFR 1.121 through inadvertence. No new matter is added by the present amendment.

Prior to calculation of the filing fee for the referenced new divisional application, kindly enter the following amendments.

AMENDMENT - CLEAN VERSIONIn the Specification:

Please insert the following new paragraph after the title on page 1 of the application:

This application hereby claims the benefit under 35 U.S.C. §120 of earlier filed U.S. Patent Application Serial No. 09/598,218 filed June 21, 2000.

Please replace the paragraph beginning "Hemoglobin is" at page 2, line 3 with the following rewritten paragraph:

Hemoglobin is the oxygen-carrying component of blood, circulated through the blood stream inside erythrocytes (red blood cells). Human normal adult hemoglobin ("Hb A") is a tetrameric protein with a molecular weight of about 64,500 containing two identical α chains having 141 amino acid residues each and two identical β chains having 146 amino acid residues each, with each also bearing prosthetic groups known as hemes. The erythrocytes help maintain hemoglobin in its reduced, functional form. The heme-iron atom is susceptible to oxidation, but may be reduced again by one of two systems within the erythrocyte, the cytochrome b_5 , and glutathione reduction systems. For a review on hemoglobin, see, Dickerson, R.E., et al. Hemoglobin: Structure, Function, Evolution, and Pathology, p. 22-24, Benjamin/Cummings, Menlo Park, CA (1983) (hereinafter "Dickerson, et al. (1983)"), the disclosure of which is incorporated herein by reference.

Please replace the paragraph beginning "The cooperative" at page 5, line 4 with the following rewritten paragraph:

The cooperative oxygenation of Hb, as measured by the Hill coefficient (" n_{max} ") is a convenient measure of its oxygenation properties. See, Dickerson, et al. (1983). Hb A has an n_{max} value of approximately 3 in its binding with O₂ under usual experimental conditions. Human abnormal Hbs with amino acid substitutions in the $\alpha_1\beta_2$ (or $\alpha_2\beta_1$) subunit interface generally result in high oxygen affinity and reduced cooperativity in O₂ binding compared to Hb A. See, for example, Dickerson, et al. (1983); Bunn, et al (1986) and Perutz, M.F., et al. Mechanisms of Cooperativity and Allosteric Regulation in Proteins pp. 19-29, Cambridge University Press (1990), the disclosure of which is incorporated herein by reference.

Please replace the paragraph beginning "The exchangeable" at page 36, line 1 with the following rewritten paragraph:

The exchangeable proton resonances of the Hb molecule arise from the exchangeable protons in the subunit interfaces. Of special interest to the present invention are the exchangeable proton resonances at 14.2, 12.9, 12.1, 11.2, and 10.7 ppm from DSS, which have been characterized as the inter-subunit H-bonds

in the $\alpha_1\beta_1$ and $\alpha_1\beta_2$ subunit interfaces in both deoxy (T) and/or oxy (R) states of Hb A (Russu, et al (1987); Fung, et al. (1975)); and Ho (1992), the disclosures of which are incorporated herein by reference). The resonances at 12.9 ppm and 12.1 ppm from DSS have been assigned to the H-bonds between $\alpha 122\text{His}$ and $\beta 35\text{Tyr}$, and $\alpha 103\text{His}$ and $\beta 131\text{Gln}$, respectively (see Russu, et al. (1987) and Simplaceanu, et al. Biophys. J. 79:1146 (2000) (hereinafter "Simplaceanu, et al. (2000)"). In the spectra of rHbCO (βN108Q) and rHbCO (αL29F , βN108Q) (as seen in Figure 6A), three resonances instead of one occur corresponding to the chemical shift of HbCO A at 12.1 ppm. The main peak occurs at 12.0 ppm, with a shoulder at 11.8 ppm and an extra resonance at 12.3 ppm. The intensities of the resonances at 12.3 and 11.8 ppm are not even 1/10 of the ones at 12.0 ppm and at 12.9 ppm, indicating that these two extra resonances are unlikely to be contributed by additional protons. The sum of the integrated areas of the resonances at 11.8, 12.0, and 12.3 ppm is about the same as the area of the single resonance at 12.9 ppm, suggesting the coexistence of three conformers of rHb (βN108Q) in CO form.

Please replace the paragraph beginning "The cooperative" at page 47, line 16 with the following rewritten paragraph:

Appropriately cross-linked rHb (βN108Q) and/or rHb (βL105W) can be incorporated into a hemoglobin-based blood